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Per- and Polyfluoroalkyl Substances: A Laboratory Primer

Moderator

Martina McGarvey, DM, Laboratory Director, Pennsylvania Department of Environmental Protection, Bureau of Laboratories, Harrisburg, PA

SPEAKERS

Jamie Strong, PhD, Chief, Human Health Risk Assessment Branch, Office of Water, Office of Science and Technology, US EPA, Washington, DC

Jody Shoemaker, PhD, Research Chemist, US EPA, Cincinnati, OH

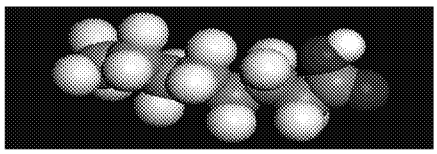
Carin Huset, PhD, Research Scientist, Public Health Laboratory, Minnesota Department of Health, St Paul, MN

EPA's Lifetime Drinking Water Health Advisories (HA) for Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA)

Jamie Strong
U.S. EPA Office of Water
Office of Science and Technology

Background-Chemical Characteristics

- PFOS and PFOA are two chemicals in a large group (hundreds) of manmade chemicals called perfluoroalkyl substances (PFAS).
- PFAS have many uses, including as surfactants and to make products more resistant to stains, grease, and water.
- PFOS and PFOA both have 8 carbon atoms and are resistant to biodegradation, photolysis and hydrolysis.
- PFOS and PFOA are the terminal degradation products formed from longer chain commercial, biodegradable precursors.
- Both chemicals have similar environmental fate and transport processes.
  - They are stable in the environment, including in water.
  - Low volatility, but adsorb to airborne particulates and can be transported long-range.
  - Mobile in water and soils.
  - Bioaccumulate across trophic levels.



Background-Physiological Behavior

- Both chemicals are very persistent in the human body.
  - PFOA half-life in blood serum: 2.3 years (general population)
  - PFOS half-life in blood serum: 5.4 years (occupational exposure)

- Six CDC National Health and Nutrition Examination Surveys (NHANES) analyzed PFAS in blood serum between 1999 and 2012.
  - PFOA and PFOS were detected in 99.7% and 99.9% of the U.S. population.
  - Serum concentrations declined over this period:
    - PFOA (geometric mean) concentration from 5.2 µg/L to 2.12 µg/L.
    - PFOS (geometric mean) concentration from 30.4 µg/L to 6.31 µg/L.

Previous and Current Uses: Industrial and Consumer Products

PFOA

- Cooking surfaces (Teflon)
- Aqueous film forming foams (used in fire fighting)
- Toothpaste, Shampoos, cosmetics
- Polishes and waxes
- Electronics
- Lubricants/surfactants/emulsifiers
- Pesticide
- Plumbing Tape
- Food containers and contact paper
- Textiles (Gore-Tex) and Leather
- Paints, varnishes, sealants
- Cleaning products
- And more…

PFOS

- Metal plating and finishing
- Fire fighting foams
- Photograph Development
- Semiconductor industry
- Aviation Fluids
- Flame repellants
- Packaging Papers
- Oil and Mining
- Stain repellants on carpets and upholstery (e.g. Stainmaster, ScotchGard)
- Cleaning products
- Paints, varnishes, sealants
- Leathers, textiles
- And more…

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Pesticide use cancelled 2008

Food packaging (pizza boxes, microwave popcorn, sandwich bags, fast food wrapping papers, etc) cancelled January 2016

Environmental Occurrence Examples

- Manufacturing sites
  - DuPont, Diakin, 3M, Ashai, Clarient, etc.
- Industrial use sites
  - Dispersion processors including Saint-Gobain sites (Hoosick Falls)
  - Glass/Cloth Coating
  - Manufacturing and Formulating Coating Products
  - Metal Coating
  - Additives
  - Film and Film Coating Manufacturing
  - Impregnated Felt Cloth
  - Fluoropolymer Fiber Production
- Industrial and municipal waste sites
  - Landfills
  - Waste water treatment plants (Industrial and Municipal)
  - Land application of biosolids
- Fire/crash training areas
  - FAA airports
  - Federal Facilities

U.S. Production

- PFOS and PFOA are the two PFAS that have been produced in the largest amounts in the U.S since 1950.
- Both PFOA and PFOS have been phased out of production in the U.S. and replaced by shorter chain PFAS or other compounds.
  - In 2000-2002, PFOS was voluntarily phased out of production in the U.S. by its primary manufacturer, 3M. EPA has issued regulations to require notification before any new future manufacturing, including importation of PFOS and its precursors. A limited set of existing uses are not subject to these regulations because they were ongoing at the time of the regulation.
  - In 2010, eight companies entered into a voluntary agreement to phase out production of PFOA and longer chain chemicals that degrade to PFOA by the end of 2015. There are notice requirements for use on imported carpets and some specialty uses are ongoing.
- Production is still ongoing in other countries, and thus, importation of products containing both compounds is possible.

Process for Development of Lifetime HAs

- **2014 Public Panel Peer Review**
  - Followed EPA's 2013 *Conflict of Interest Review Process for Contractor-Managed Peer Reviews of EPA HISA and ISI Documents.*
    - Three Federal Register Notices: 1) released draft documents for 60-day public comment period and solicited panel nominations, 2) published interim list of panel members for public comment, and 3) announced final panel and meeting details.
  - Panel included experts with expertise in epidemiology, toxicology (liver, immune, neurological and reproductive and developmental effects, risk assessment, pharmacokinetic modeling, and mode-of-action.
  - Public comments on the draft documents were provided to the panel prior to the panel meeting in August 2014.

Summary of Health Effects

- PFOA and PFOS health effects information is available from animal studies and human epidemiology studies.
- Studies indicate that PFOA and PFOS exposure results in multiple health effects including: developmental effects, effects on serum lipids and total cholesterol, liver and kidney effects, immune effects, reproductive effects, and cancer.
- Animal studies were used quantitatively to develop candidate non cancer reference doses (RfDs).
  - Human epidemiology studies were used as additional supporting lines of evidence.
- Under EPA's Cancer Guidelines there is *Suggestive* evidence of carcinogenic potential for both PFOA and PFOS.
  - PFOA-Positive association for kidney and testicular cancers from epidemiology literature and liver, testicular, and pancreatic tumors in rats.
  - PFOS-No positive associations from epidemiology literature and evidence of liver adenomas and thyroid in rats (lacked dose-response).

Reference Dose (RfD) Selection

- EPA modeled average serum values using a peer-reviewed pharmacokinetic model (rat, mouse, and monkey) developed by ORD.
  - **PFOA:** 6 studies for effects on development (delayed ossification and accelerated puberty, pup body weight; adult body and kidney weight); liver; and immune system.
  - **PFOS:** 6 studies for effects on development (pup body weight, neurodevelopment, pup survival) and liver.

- For both PFOA and PFOS, the RfDs based on multiple adverse effects resulting from short-term and longer-term exposures fall within a narrow range.

- EPA selected the most sensitive RfD based on developmental effects to calculate a health advisory protective for the general population and sensitive lifestages.

Critical Studies Selected as Basis for RfDs

PFOA

- Lau et al., 2006
  - Developmental toxicity study
  - Dosing throughout pregnancy gestational days 1-17; pups sacrificed at weaning (e.g., lactational exposure included)
- Decreased ossification in proximal phalanges and accelerated puberty in male pups
- RfD derived from a lowest observed adverse effect level (LOAEL) and a total uncertainty factor of 300
- **RfD = 0.00002 mg/kg/d**

PFOS

- Luebker et al., 2005b
  - 2-generation reproductive toxicity study
  - Dosing premating and throughout pregnancy and lactation for 2 generations
- Decreased body weight and weight gain in pups
- RfD derived from a no observed adverse effect level (NOAEL) and a total uncertainty factor of 30
- **RfD = 0.00002 mg/kg/d**

Lifetime HA Calculation for PFOA and PFOS

$$\text{Lifetime HA} = \frac{\text{RfD} \times \text{RSC}}{\text{DWI/BW}}$$

Where:

HA = Health Advisory

RfD = Reference Dose [0.00002 mg/kg/d]

RSC = Relative Source Contribution [20%]

DWI/ BW = DWI adjusted by BW for lactating women [0.054 L/kg]

$$\text{Lifetime HA} = \frac{0.00002\ \text{mg/kg/d} \times 0.2}{0.054\ \text{L/kg}}$$

Lifetime HA = 0.00007 mg/L

= 0.07 µg/L or 70 parts per trillion (ppt)

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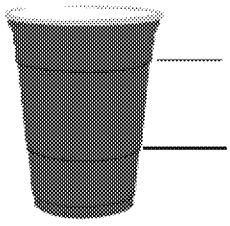
Due to the potential increased susceptibility during the time period of pregnancy and lactation, EPA used drinking water intake and body weight parameters for lactating women in the calculation of a Lifetime HA for this target population during this potential critical time period. EPA used the rate of 54 mL/kg-day representing the consumers only estimate of combined direct and indirect community water ingestion at the 90th percentile for lactating women (see Table 3-81 in U.S EPA, 2011). Comparing between the pregnant and lactating woman, the lactating woman is the most sensitive given her increased water intake rate (54 mg/L-day) to support milk production. Additionally, human studies have shown that PFOA and PFOS are transferred from mother to infant via cord blood and breast milk. A recent study showed that breast milk contributed > 94% and > 83% of the total PFOS and PFOA exposure, respectively, in 6-month-old infants (Haug et al., 2011).

Relative Source Contribution (RSC)



RSC of 80%-Exposure is primarily from drinking water; reserve 20% of RfD to account for exposure through other sources (e.g., dust, air, soil, etc.)

RSC of 20%-Exposure is primarily through other sources (e.g., dust, air, soil, etc.); reserve 20% of RfD to account for exposure via drinking water.

- EPA derived an **RSC of 20% for PFOA and PFOS** for the national HA based on available occurrence information and considering the environmental persistence of these compounds
  - CDC data provide evidence of broad exposure to PFAS from multiple sources.
  - Currently, diet is the major source of PFOA and PFOS:
    - Food products including fish, snack foods, vegetables grown in contaminated soils, and meat and dairy products from exposed grazing animals
    - Food packaging products and use of Teflon cookware
  - Contaminated drinking water
  - Indoor dust is another major source (especially to children) from treated carpets and furniture/textiles in homes, offices, automobiles.
  - Other sources of legacy exposure or exposure to precursors: soils, air, clothing, cosmetics, cleaning materials, etc.

Lifetime HA and Application

- The Lifetime HAs are based on developmental effects resulting from exposures that occur during pregnancy and lactation (nursing) and are protective for all other health effects (non-cancer and cancer) that may occur during a lifetime of exposure to these chemicals in drinking water.

- Because the critical effects identified are developmental effects and can potentially result from a short-term exposure during a critical period of development, the Lifetime HAs apply to both short term (weeks to months), such as the time periods during pregnancy and nursing and bottle feeding, as well as chronic (lifetime) exposure scenarios.

- Because the toxicological effects of PFOA and PFOS are very similar, where these chemicals co-occur in drinking water at the same time, we recommend that the HA be applied to the sum of the concentrations of PFOA and PFOS.
  - EPA has not evaluated the toxicity of other PFAS at this time.

- EPA Office of Water PFOA and PFOS website: https://www.epa.gov/ground-water-and-drinking-water/drinking-water-health-advisories-pfoa-and-pfos

- Joyce Donohue, Chemical Manager for PFOA and PFOS: donohue.joyce@epa.gov

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APHL Webinar-2016

Implementing EPA Method 537

*Jody A. Shoemaker, Paul E. Grimmett, Brenda K. Boutin**

Office of Research and Development
National Exposure Research Laboratory

* National Council on Aging

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Background

- EPA Method 537 was developed for PFAAs in response to heightened interest by the public and the scientific community over these chemicals of emerging concern
- In 2009, PFOS and PFOA were placed on the Contaminant Candidate List 3 (CCL 3)
- Revision 1.1 of Method 537 was released September 2009

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Method 537: SPE-LC/MS/MS
14 Perfluorinated Alkyl Acids (PFAA)

Perfluorocarboxylic acids (9) **Perfluorosulfonic acids (3)**
Perfluorosulfonamidoacetic acids (2)

Method Analytes in UCMR 3

PFHpA – perfluoroheptanoic acid
PFOA – perfluorooctanoic acid
PFNA – perfluorononanoic acid
PFBS – perfluorobutanesulfonic acid
PFHxS – perfluorohexanesulfonic acid
PFOS – perfluorooctane sulfonic acid

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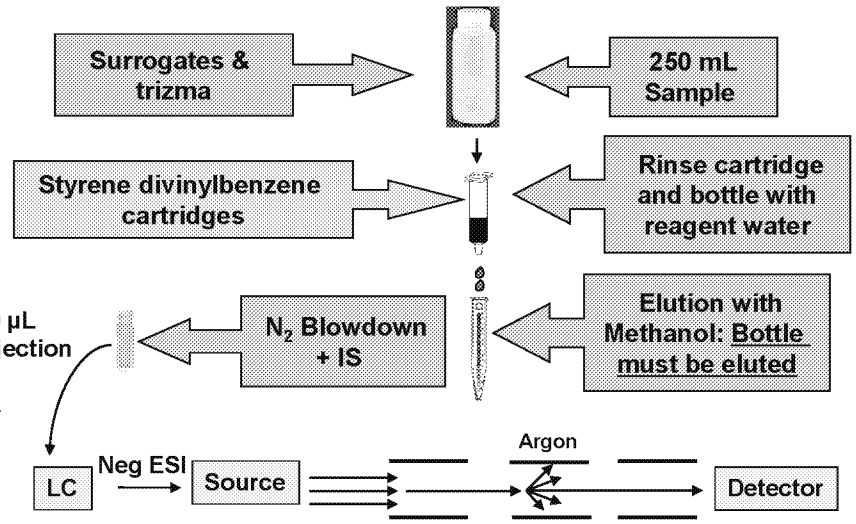
Method 537 Approach

Preservative
trizma

Surrogates
^{13}C-PFHxA
^{13}C-PFDA
d_5-NEtFOSAA

Internal Standards
^{13}C-PFOA
^{13}C-PFOS
d_3-NMeFOSAA



LC/MS/MS - Selected Reaction Monitoring

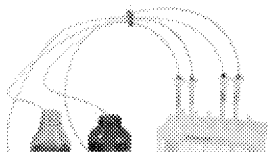
Method 537 Implementation Suggestions

United States Environmental Protection Agency

General:

- wide range of water solubilities (C_4-C_{14}) which can affect recoveries through adsorption or extraction losses

SPE:



- Method recommends use of a polypropylene transfer tube system, which transfers the sample directly from the sample container to the SPE cartridge.
- PFBS Recovery – may be adversely affected if SPE loading flow rate is too high
- Rinsing the sample bottle with the SPE elution solvent is required and is critical for recovery of the PFAAs with ≥8 carbon chains

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Method 537 Implementation Suggestions

Laboratory and field blank contamination:

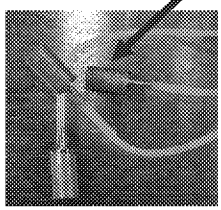
- Many lab supplies and equipment can contain PFAAs.
- Personnel must be aware of potential PFAA contamination consumer products and take measures to avoid these sources of PFAAs.
- Section 8.3.1 requires a field reagent blank (FRB), Sampler must open the shipped FRB in the field and pour the preserved reagent water into the empty shipped sample bottle and seal the FRB. Ensures PFAAs were not introduced into the sample during sample collection/handling or from preservatives and bottles.

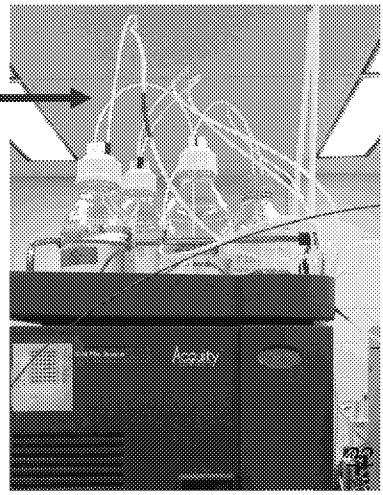
Method 537 Implementation Suggestions

United States Environmental Protection Agency

LC:

- Polypropylene (PP) vials/caps are necessary to prevent contamination of the sample from PTFE coated septa.
  - ✓ PP caps do not re-seal, so evaporation occurs after injection.
  - ✓ Multiple injections from the same vial are not possible.
  - ✓ Suggest splitting extract before injection
- PFAAs can build up in the PTFE solvent transfer lines on the LC during inactivity.
  - ✓ Recommend replacing PTFE lines with PEEK tubing
  - ✓ Recommend replacing PTFE solvent frits with stainless steel frits





Method 537 Implementation Suggestions

United States Environmental Protection Agency

LC: (cont.)

- Modifications to LC conditions should still produce conditions such that co-elution of method analytes is minimized to reduce the probability of suppression/enhancement effects.
- PFAAs, from LC system components and mobile constituents, will build up on the head of the LC column during mobile phase equilibration.
  - ✓ Keep post-equilibration time constant and as short as possible

5 min post-equilibration

LC Gradient		
Time (min)	% 20 mM ammonium acetate	% Methanol
Initial	60.0	40.0
1.0	60.0	40.0
25.0	10.0	90.0
32.0	10.0	90.0
32.1	60.0	40.0
37.0	60.0	40.0

Modifications to Method 537

"modified" Method 537: this terminology leads to confusion.

- ✓ Method 537 was developed and multi-lab verified for the sole purpose of analyzing the method analytes in **drinking water.**
- ✓ Method 537 allows limited flexibility as defined in Section 1.6.
- ✓ If modifications are made outside the allowed flexibility, then the procedure is no longer Method 537 and should not be considered "modified" Method 537. These modifications have not been verified.
- ✓ Other matrices may require additional clean up steps.
- ✓ Recommend that all PFAA methods adhere to the QC control samples required in Method 537 to avoid losses in data integrity.

United States Environmental Protection Agency

EPA Technical Advisory - Laboratory Analysis of Drinking Water Samples for PFOA Using EPA M537 Rev. 1.1

EPA has recently learned that laboratories have identified different approaches for implementation of EPA Method 537 Rev 1.1 for analysis of PFOA.

- Some laboratories have analyzed PFOA by quantitation of only the linear isomer while others have quantified both linear and branched-chain isomers to determine the concentration of PFOA.

- The linear isomer represents the predominant form of PFOA, but samples may also have some degree of branched-chain isomers.

https://www.epa.gov/sites/production/files/2016-09/documents/pfoa-technical-advisory.pdf

United States Environmental Protection Agency

EPA Technical Advisory – continued

How should laboratories quantitate PFOA using EPA Method 537?

To account for linear and branched isomers of PFOA, EPA recommends that integration and quantitation of real-world drinking water samples include peaks that represent both linear and branched isomers.

- There is currently no certified quantitative PFOA standard that contains both linear and branched isomers, thus EPA recommends labs calibrate instrumentation using a certified quantitative standard containing only the linear isomer.
- Identify the branched isomers by analyzing a "qualitative/semi-quantitative" PFOA mixed standard that includes both linear and branched isomers and compare retention times and MS/MS transitions.
- Quantitate PFOA by integrating the total response (linear + branched isomers) and relying on the initial calibration with the linear-isomer quantitative standard.

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Conclusions

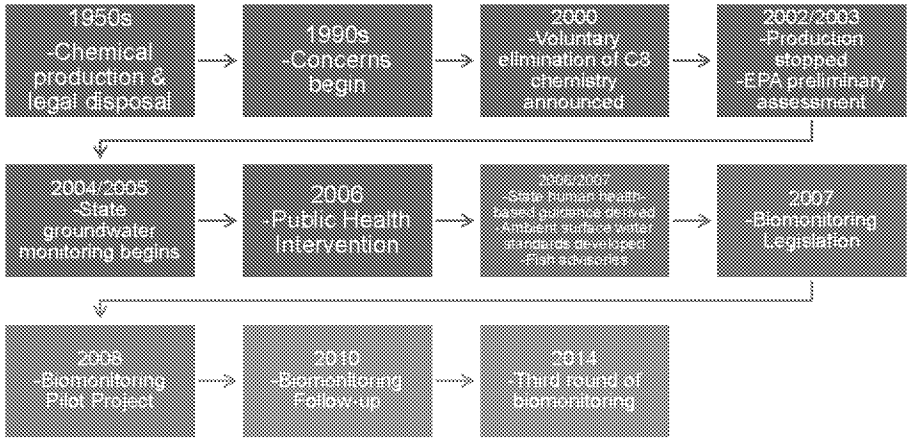
- Implementation of Method 537 for drinking water analyses requires
  - ✓ Careful attention to avoid lab sources of PFAA compounds
  - ✓ Minimize instrument sources of PFAA
  - ✓ Following SPE steps carefully to avoid adsorption losses or inadvertent extraction losses
  - ✓ Following all QC practices required in Method 537

PFAS in Minnesota: A State PHL Perspective

Carin Huset, PhD

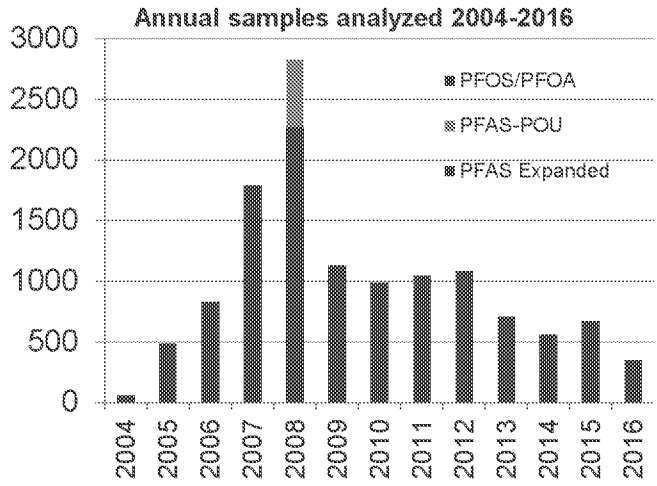
October 17, 2016

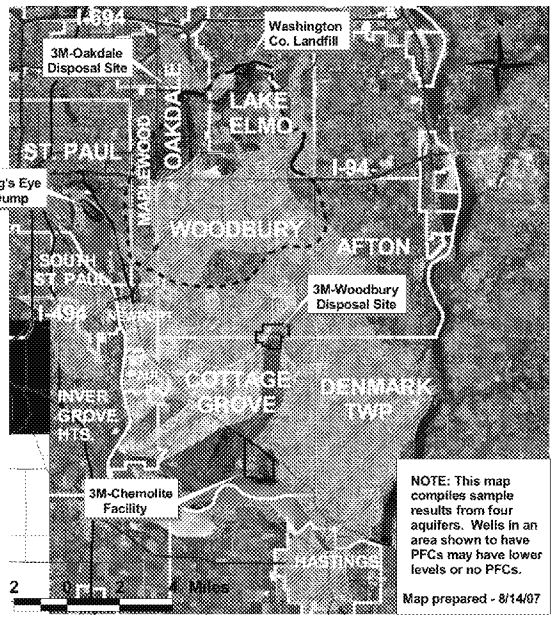
History of PFASs in Minnesota



Drinking Water Analysis

- LC/MS/MS Method
  - Direct injection
  - Reversed phase chromatography
  - Isotope dilution quantitation
  - Report levels:
    - 2007: 300ng/L
    - 2016: 25-50ng/L
  - Method Performance
    - Precision
      - 2-7%
    - Accuracy
      - 99-106%





PFCs in the Southeast Metro Area

- PFCs not detected
- PFBA only, 0.2 - 0.5 ppb
- PFBA only, 0.6 - 0.9 ppb
- PFBA only, 1.0 - 1.5 ppb
- PFBA only, 1.6 - 1.9 ppb
- PFBA only, > 2.0 ppb
- Multiple PFCs detected
- Approximate boundary of northern plumes, dashed where uncertain

Minnesota Environmental Health Tracking and Biomonitoring Legislation (2007)

- Establish Environmental Health Tracking and Biomonitoring Program
- Conduct a pilot biomonitoring program of 4 projects
  - Include 2 communities "likely to be exposed" to PFAS
  - Required inclusion of PFBA
- Created Scientific Advisory Panel

Biomonitoring Projects in 2008, 2010, 2014

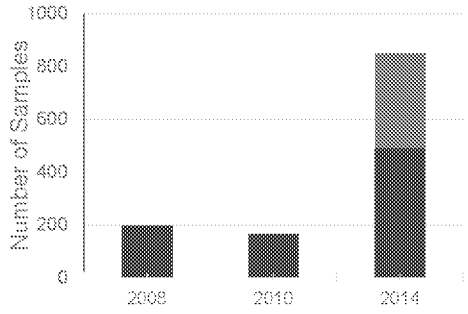
- Participant recruitment
  - Randomly sampled from water billing records
  - Required residence prior to 2005
- 149 residents participated in all 3 studies
  - Additional cohort of 156 new residents recruited in 2014
- Questionnaires used to get more data on exposure routes
- Local clinic used to collect and process specimens
  - MDH lab trained clinic for collection of serum
  - PFAS analysis at MDH

Serum Analysis

Method used in 2008 & 2010
- Based on Kuklenyik, 2004
- 1mL serum
- SPE cartridges

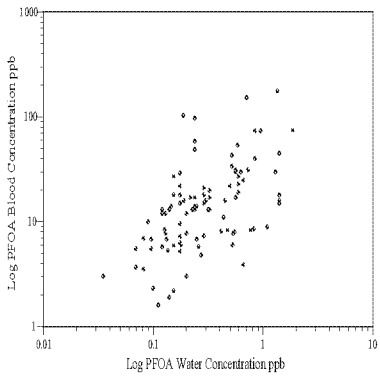
Method used in 2014
- Similar to Flaherty, 2005
- 400uL serum
- Protein precipitation, 96 well plate

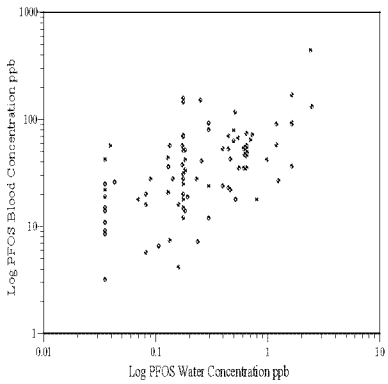
- Method Performance
  - Archived 2008 samples used to validate 2014 method
    - ±20% RPD
  - Precision and Accuracy
    - 2008/2010: 2-8%, 100-115%
    - 2014: 1-8%, 99-115%
- Both methods
  - Matrix matched calibration curves and isotope dilution
  - Reversed phase HPLC/MS/MS
  - Report level: 0.1 ng/mL



2008 Blood Levels Related to Drinking Water Levels

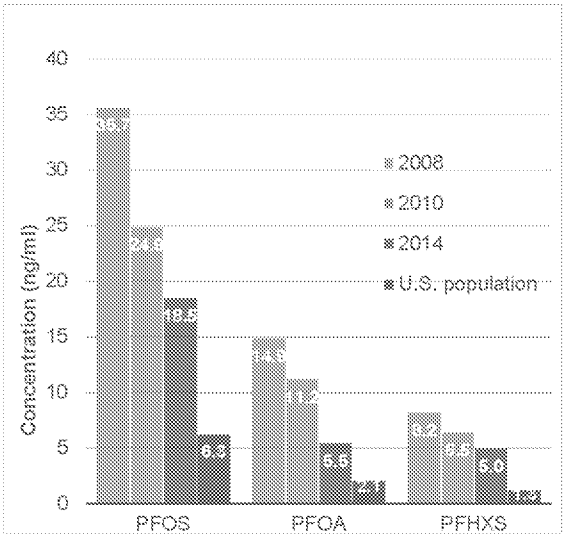
- Analysis of a subset of private well users (n=98)





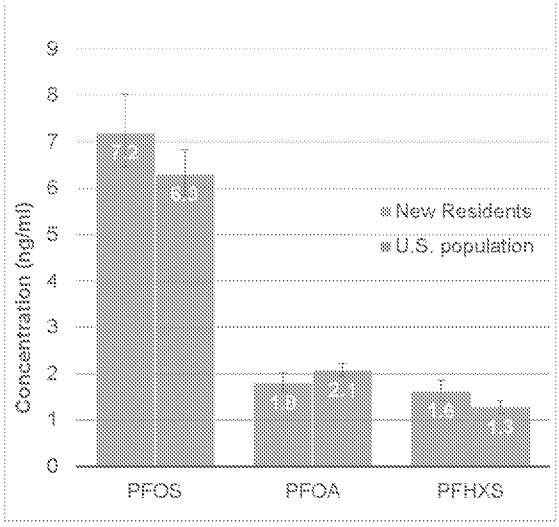
2008, 2010, 2014 Blood PFAS levels compared to NHANES

- Blood levels of long term residents on the decline
- Mean individual percent change 2008-2014 (n=149)
  - PFOS 45% decrease
  - PFOA 59% decrease
  - PFHxS 34% decrease
- Intervention was effective to reduce exposure



PFAS in New Residents (2014)

- New residents (n=156)
  - Moved to community after intervention
- Compared to NHANES 2011-2012
  - No significant difference in levels between groups



Summary

- Drinking water analysis is ongoing
- Biomonitoring projects answered questions from community and legislature
  - PFAS blood levels declining in population with prior exposure
  - PFAS blood levels in new residents comparable to US population
  - Mixed success in teasing out sources of exposure

Acknowledgements

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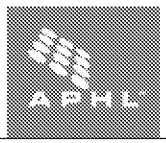
  - Julie Kadrie, MPH — Health Risk Communications
  - Mike Convery, PG — Hydrogeologist
- MDH PHL
  - Marty Bevan, PhD — Research Scientist
  - Kitrina Barry, MS — Research Scientist

For More Information

- PFAS in Minnesota
  - http://www.health.state.mn.us/divs/eh/hazardous/topics/pfcs/index.html
- Minnesota Environmental Health Tracking and Biomonitoring Program
  - http://www.health.state.mn.us/divs/hpcd/tracking/biomonitoring/index.html

References:
Kuklenyik et al, Environmental Science and Technology, 2004, 28, 3698-3704
Flaherty et al, Journal of Chromatography B, 2005, 819, 329-338
Landsteiner et al, Journal of Environmental Health, 2014, 77(5), 14-19



Questions